



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Atty. Docket No: 081356-0253

In re patent application of

NAKASHIMA, NOBUTAKA *et al.*

Serial No.: 10/553,979

Filed: October 20, 2005

For: METHOD OF PRODUCING RECOMBINANT PROTEIN IN BACTERIUM BELONGING TO GENUS
RHODOCOCCUS

STATEMENT TO SUPPORT FILING AND SUBMISSION IN
ACCORDANCE WITH 37 C.F.R. §§ 1.821-1.825

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450
Mail Stop SEQUENCE

Sir:

In connection with a Sequence Listing submitted concurrently
herewith, the undersigned hereby states that:

1. the submission, filed herewith in accordance with 37 C.F.R. §
1.821(g), does not include new matter;

2. the content of the electronically filed Sequence Listing is
submitted in accordance with 37 C.F. § 1.821(e).

Respectfully submitted,

10 September 2008
Date

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4829485_1.TXT
SEQUENCE LISTING

<110> NAKASHIMA, NOBUTAKA
TAMURA, TOMOHIRO

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BELONGING TO GENUS RHODOCOCCLUS

<130> 081356-0253

<140> 10/553,979

<141> 2005-10-20

<150> PCT/JP04/005585

<151> 2004-04-19

<150> JP 2003116280

<151> 2003-04-21

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<170> PatentIn Ver. 3.3

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<213> Artificial Sequence

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<223> Description of Artificial Sequence: Synthetic
vector pTip-NH2 sequence

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 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic
 vector pTip-RT2 sequence

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<212> DNA

<213> Artificial Sequence

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<223> Description of Artificial Sequence: Synthetic
vector pTip-QC1 sequence

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<212> DNA

<213> Artificial Sequence

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vector pNit-QT1 sequence

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<210> 101

<211> 6058

<212> DNA

<213> Artificial Sequence

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<223> Description of Artificial Sequence: Synthetic
vector pNit-RT1 sequence

<400> 101

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<223> Description of Artificial Sequence: Synthetic
vector pNit-RT2 sequence

<400> 102

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<223> Description of Artificial Sequence: Synthetic
vector pNit-QC1 sequence

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vector pNit-RC1 sequence

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<213> Rhodococcus erythropolis

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<223> mutated TipA gene promoter

<400> 107

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<210> 108

<211> 422

<212> DNA

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<220>

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<222> (151)..(222)

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[illegible]

<210> 109
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<212> PRT
<213> Artificial Sequence

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<220>
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      peptide
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<212> DNA
<213> Artificial Sequence
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<220>
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<210> 111
<211> 416
<212> DNA
<213> Artificial Sequence
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<220>
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<220>

<221> CDS

<222> (151)..(216)

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gcgtggacgg cgtcagagaa gggagcggcc atg gga att cta cgt agc ggc cgc 174
Met Gly Ile Leu Arg Ser Gly Arg

gga tcc aag ctt aga tct cga gga cat cac cat cac cat cac 216
Gly Ser Lys Leu Arg Ser Arg Gly His His His His His His
10 15 20

tgaactagtc gacccaccgg caccctgag cccctcgctg cgggtgccgg tgcgaqqqac 276

tgcaacacgc gaaacctgca caaacacacg gaggttggaa tgagcgccac qgacacaccc 336

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cgcctcagcg ggactctagt 416

$\langle 210 \rangle$ 112

<211> 22

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic peptide

$\langle 400 \rangle$ 112

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His His His His His His
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<210> 113

<211> 42

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic nucleotide sequence

<400> 113

gtctagaaat aattttgttt aactttaaga aggagatata cc 42

<210> 114

<211> 425

<212> DNA

<213> Artificial Sequence

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<223> Description of Artificial Sequence: Synthetic nucleotide sequence

4829485_1.TXT

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gcgtggacgg cgtcagagaa gggagcgc atg ggc cat cac cat cac cat cac 174
Met Gly His His His His His His
1 5
gcc atg gga att cta cgt agc ggc cgc gga tcc aag ctt aga tct cga 222
Ala Met Gly Ile Leu Arg Ser Gly Arg Gly Ser Lys Leu Arg Ser Arg
10 15 20
gga tgaactagtc gaccacccgg caccctgag ccctcgtg cgggtgccgg 275
Gly
25
tgcgagggac tgcaacacgc gaaacctgca caaacacacg gaggttgaa tgagcgccac 335
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cctgctacgc cgcctcagcg ggactctagt 425

<210> 115
<211> 25
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 115
Met Gly His His His His His His Ala Met Gly Ile Leu Arg Ser Gly
1 5 10 15
Arg Gly Ser Lys Leu Arg Ser Arg Gly
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<210> 116
<211> 43
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic nucleotide sequence

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gtctagaaat aattttgttt aactttaaga aggagatata cat 43

<210> 117
<211> 416
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic nucleotide sequence

<220>

<221> CDS

<222> (151) .. (216)

<400> 117

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gcgtggacgg cgtcagagaa gggagcgcac atg gga att cta cgt agc ggc cgc 174
Met Gly Ile Leu Arg Ser Gly Arg
1 5

gga tcc aag ctt aga tct cga gga cat cac cat cac cat cac 216
Gly Ser Lys Leu Arg Ser Arg Gly His His His His His His
10 15 20

tgaactagtc gacccaccgg caccctgag cccctcgctg cgggtgccgg tgcgagggac 276

tgcaacacgc gaaacctgca caaacacacg gaggttggaa tgagcggcac ggacacaccc 336

gataaccggcg ccgttccacc ccggttggtg accaccgctg gggcggctga cctgctacgc 396

cgccctcagcg ggactctagt 416

<210> 118

<211> 22

<212> PRT

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<220>

<223> Description of Artificial Sequence: Synthetic peptide

<400> 118

Met Gly Ile Leu Arg Ser Gly Arg Gly Ser Lys Leu Arg Ser Arg Gly
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His His His His His His
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<210> 119

<211> 43

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic nucleotide sequence

<400> 119

gtctagaaat aattttgttt aactttaaga aggagatata cat 43

<210> 120

<211> 81

<212> DNA

<213> Artificial Sequence

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<220>

<221> CDS

<222> (3)..(68)

<400> 120

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  Met Gly Ile Leu Arg Ser Gly Arg Gly Ser Lys Leu Arg Ser Leu
    1           5           10           15

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gag cat cac cat cac cat cac tgaactagtc gac      81
Glu His His His His His His
                20

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<210> 121

<211> 22

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic peptide

<400> 121

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Met Gly Ile Leu Arg Ser Gly Arg Gly Ser Lys Leu Arg Ser Leu Glu
  1           5           10           15

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His His His His His His
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<210> 122

<211> 82

<212> DNA

<213> Artificial Sequence

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<223> Description of Artificial Sequence: Synthetic nucleotide sequence

<220>

<221> CDS

<222> (4)..(69)

<400> 122

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cat atg gga att cta cgt agc ggc cgc gga tcc aag ctt aga tct ctc      48
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    1           5           10           15

```

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gag cat cac cat cac cat cac tgaactagtc gac      82
Glu His His His His His His
                20

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<210> 123

<211> 22

<212> PRT

<213> Artificial Sequence

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<223> Description of Artificial Sequence: Synthetic peptide

<400> 123

Met Gly Ile Leu Arg Ser Gly Arg Gly Ser Lys Leu Arg Ser Leu Glu
1 5 10 15His His His His His His
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<210> 124

<211> 22

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic nucleotide sequence

<400> 124

gtcagagaag ggagcggcca tg

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<210> 125

<211> 45

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic nucleotide sequence

<400> 125

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45

<210> 126

<211> 14

<212> PRT

<213> Rhodococcus erythropolis

<400> 126

Gly Leu Arg Ser Cys Gly Lys Gly Trp Ile Cys Pro Cys Cys
1 5 10

<210> 127

<211> 8

<212> PRT

<213> Rhodococcus erythropolis

<400> 127

Met Val Thr Met Thr Met Arg His
1 5

<210> 128

<211> 26

<212> PRT

<213> Rhodococcus erythropolis

<400> 128

Gly Cys Asp Gly Tyr Val Arg Ala Val Glu Ile Thr His Gly Lys Asn
1 5 10 15Gly Trp His Val His Val His Ala Leu Leu
20 25

<210> 129

<211> 10

<212> PRT

<213> Rhodococcus erythropolis

<400> 129

Leu Ala Ala Tyr Leu Thr Lys Ile Ala Ser
1 5 10

<210> 130

<211> 21

<212> PRT

<213> Rhodococcus erythropolis

<400> 130

Trp Arg Glu Phe Glu Phe Gly Ser Met Gly Arg Arg Ala Ile Ala Trp
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<210> 131

<211> 14

<212> PRT

<213> Arcanobacterium pyrogens

<400> 131

Gly Leu His Thr Cys Gly Ser Val Trp Ala Cys Pro Val Cys
1 5 10

<210> 132

<211> 8

<212> PRT

<213> Arcanobacterium pyrogens

<400> 132

Met Leu Thr Leu Thr Gln Arg His
1 5

<210> 133

<211> 26

<212> PRT

<213> Arcanobacterium pyrogens

<400> 133

Gly Leu Val Gly Tyr Val Arg Ala Asn Glu Ile Thr His Gly Lys His
1 5 10 15

Gly Trp His Val His Ser His Val Leu Ile
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<210> 134
 <211> 10
 <212> PRT
 <213> Arcanobacterium pyrogens

<400> 134
 Ile Gly Asn Tyr Val Ser Lys Met Gln Thr
 1 5 10

<210> 135
 <211> 21
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 <213> Arcanobacterium pyrogens

<400> 135
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 1 5 10 15

Ser Lys Gly Leu Arg
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<210> 136
 <211> 8
 <212> PRT
 <213> Brevibacterium lactofermentum

<400> 136
 Met Phe Val Gly Thr Val Arg His
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<210> 137
 <211> 26
 <212> PRT
 <213> Brevibacterium lactofermentum

<400> 137
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 1 5 10 15

Gly Trp His Leu His Arg Asn Met Leu Leu
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<210> 138
 <211> 10
 <212> PRT
 <213> Brevibacterium lactofermentum

<400> 138
 Met Ala Thr Tyr Leu Ala Lys Gly Met Ser
 1 5 10

<210> 139
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 <212> PRT

<213> Brevibacterium lactofermentum

<400> 139

Trp Arg Glu Tyr Glu Val Gly Ser Lys Asn Leu Arg Ser Ser Trp Ser
1 5 10 15

Arg Gly Ala Lys
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<210> 140

<211> 14

<212> PRT

<213> Streptomyces phaeochromogenes

<400> 140

Gly Leu Val Arg Cys Gly Arg Ile Trp Phe Cys Pro Glu Cys
1 5 10

<210> 141

<211> 8

<212> PRT

<213> Streptomyces phaeochromogenes

<400> 141

Leu Val Thr Phe Thr Ala Arg His
1 5

<210> 142

<211> 27

<212> PRT

<213> Streptomyces phaeochromogenes

<400> 142

Gly Tyr Ile Gly Met Val Arg Ala Ala Glu Val Thr Arg Ser Lys Lys
1 5 10 15

Asn Gly Tyr His Pro His Leu Asn Leu Leu Val
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<210> 143

<211> 10

<212> PRT

<213> Streptomyces phaeochromogenes

<400> 143

Leu Ile Glu Tyr Leu Thr Lys Asn Gln Asp
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<210> 144

<211> 21

<212> PRT

<213> Streptomyces phaeochromogenes

<400> 144

Trp Ala Gln Tyr Glu Glu Ala Leu Ala Gly Arg Arg Ala Ile Glu Trp
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Thr Arg Gly Leu Arg

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<210> 145
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 <213> Streptomyces lividans

<400> 145
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<210> 146
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 Leu Val Thr Phe Thr Ala Arg His
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<210> 147
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 20 25

<210> 148
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 <213> Streptomyces lividans

<400> 148
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 1 5 10

<210> 149
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 <213> Streptomyces lividans

<400> 149
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 1 5 10 15
 Thr Arg Tyr Leu Arg
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<210> 150
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 <212> PRT
 <213> Streptomyces nigrifaciens

<400> 150

Gly Leu Met Arg Cys Gly Arg Ile Trp Leu Cys Pro Val Cys
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<210> 151

<211> 8

<212> PRT

<213> Streptomyces nigrifaciens

<400> 151

Leu Val Thr Phe Thr Ala Arg His
1 5

<210> 152

<211> 26

<212> PRT

<213> Streptomyces nigrifaciens

<400> 152

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<210> 153

<211> 10

<212> PRT

<213> Streptomyces nigrifaciens

<400> 153

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<210> 154

<211> 21

<212> PRT

<213> Streptomyces nigrifaciens

<400> 154

Trp His Glu Tyr Glu Arg Ala Thr Lys Gly Arg Arg Ala Ile Glu Trp
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<210> 155

<211> 30

<212> DNA

<213> Rhodococcus erythropolis

<400> 155

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30

<210> 156

<211> 27

<212> DNA

<213> *Arcanobacterium pyrogens*

<400> 156

caggatatgcg gaaaacttta ggaacaa 27

<210> 157

<211> 32

<212> DNA

<213> *Brevibacterium lactofermentum*

<400> 157

gaaatagaag tgaacacctc taaggaaccg ca 32

<210> 158

<211> 31

<212> DNA

<213> *Streptomyces phaeochromogenes*

<400> 158

ctggcaaaaa gggacgccta ggtaaagggt t 31

<210> 159

<211> 30

<212> DNA

<213> *Streptomyces lividans*

<400> 159

gaggcaaaag cgaacacctt gggaaagaaa 30

<210> 160

<211> 32

<212> DNA

<213> *Streptomyces nigrifaciens*

<400> 160

gacccaaaac gtgtcgcgcc ttgggaaaga aa 32

<210> 161

<211> 270

<212> DNA

<213> *Rhodococcus erythropolis*

<400> 161

tgagggcatc	cccccgatac	ttgccgcttt	gaagctgggt	gtctctctgt	cagggctgcg	60
atagcaccgc	gtagcggctt	ggccttgaca	gagagacggc	ctgtttcatg	gttgggtctc	120
gggggctgac	cgggcagata	gaaaaaggcc	ggccgatttg	gctgccgact	atTTTTgcag	180
gtaaaccat	ctcatgagca	tcaatgaacg	tcccgttgta	tcgcagcgcg	tcagagcttc	240
gtagacgtcg	atggcgttgt	gatgggtgtg				270

<210> 162

<211> 170

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

nucleotide sequence

<400> 162
 tgtacatatc gaggcgggct cccacggccg cccgggctga gggagccgac ggcacgcggc 60
 ggctcacggc gtggcacgcg gaacgtccgg gcttgacac ctcgtcacgt gaggaggcag 120
 cgtggacggc gtctagaaat aattttgttt aactttaaga agaagatata 170

<210> 163
 <211> 95
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic
 nucleotide sequence

<220>
 <221> CDS
 <222> (3)..(92)

<400> 163
 cc atg ggc cac cat cac cat cac cat atg gga att cta cgt agc ggc 47
 Met Gly His His His His His His Met Gly Ile Leu Arg Ser Gly
 1 5 10 15
 cgc gga tcc aag ctt aga tct ctc gag cat cac cat cac cat cac tga 95
 Arg Gly Ser Lys Leu Arg Ser Leu Glu His His His His His His
 20 25 30

<210> 164
 <211> 30
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic
 peptide

<400> 164
 Met Gly His His His His His His Met Gly Ile Leu Arg Ser Gly Arg
 1 5 10 15
 Gly Ser Lys Leu Arg Ser Leu Glu His His His His His His
 20 25 30

<210> 165
 <211> 99
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic
 nucleotide sequence

<220>
 <221> CDS
 <222> (4)..(96)

<400> 165
 cat atg ggc cat cac cat cac cat cac gcc atg gga att cta cgt agc 48
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Met Gly His His His His His His Ala Met Gly Ile Leu Arg Ser
1 5 10 15

ggc cgc gga tcc aag ctt aga tct ctc gag cat cac cat cac cat cac 96
Gly Arg Gly Ser Lys Leu Arg Ser Leu Glu His His His His His His
20 25 30

tga 99

<210> 166

<211> 31

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic peptide

<400> 166

Met Gly His His His His His His Ala Met Gly Ile Leu Arg Ser Gly
1 5 10 15

Arg Gly Ser Lys Leu Arg Ser Leu Glu His His His His His His
20 25 30

<210> 167

<211> 197

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic nucleotide sequence

<400> 167

actagtcgac ccaccggcac ccgtgagccc ctcgctgcgg gtgccggtgc gagggactgc 60
aacacgcgaa acctgcacaa acacacggag gttggaatga gcgccacgga cacacccgat 120
accggcgccg ttccaccccg gttggtgacc accgctgggg cggtgacct gctacgccgc 180
ctcagcggga ctctagt 197

<210> 168

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic 6xHis tag

<400> 168

His His His His His His
1 5

<210> 169

<211> 147

<212> DNA

<213> Artificial Sequence

<220>

4829485_1.TXT

<223> Description of Artificial Sequence: Synthetic
nucleotide sequence

<400> 169

cgcccgggct gagggagccg acggcacgcg gcggctcacg gcgtggcacg cggaacgtcc 60
gggcttgac ctcacgtcac gtgaggaggt ataatggacg gcgtctagaa ataattttgt 120
ttaactttaa gaaggagata taccatg 147

<210> 170

<211> 124

<212> DNA

<213> Rhodococcus erythropolis

<220>

<223> mutated TipA gene promoter

<400> 170

cgcccgggct gagggagccg acggcacgcg gcggctcacg gcgtggcacg cggaacgtcc 60
gggcttgac ctcacgtcac gtgaggaggt ataatggacg gcgtcagaga agggagcggc 120
catg 124